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August 7, 2000

MEMORANDUM

DP Barcode: 251310

SUBJECT: CGA 302371 Method: Evaluation Report No. ECM 0162S1

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The BEAD/Environmental Chemistry Lab has performed an Environmental Chemistry Method Evaluation (ECME) on CGA 302371 (metabolite of CGA 184927) in soil using the method, "Method of Analysis for the Determination of CGA 302371 (UE Metabolite) in soil by LC/MS".

The attached method evaluation report includes three parts:

Part I: Summary and Conclusions

In this section any problems encountered with the method and how they were handled are discussed. ECL's opinion of how well the method performed is also presented.

Part II: Analytical Results

In this section the individual results of each sample at each spiking level of each analyte is listed. The arithmetical means and descriptive statistics for each spiking level are also presented here.

Part III: Experimental Details

In this section any modification(s) that were made to the method, along with instrument parameters, spiking levels, example calculations, representative samples and standard chromatograms and standard curves are listed and/or discussed.

If you have questions concerning this report, please contact Bob Maxey at (228) 688-1225 or Aubry Dupuy at (228) 688-3212.

cc: Christian Byrne, QA Officer
BEAD/ECL

Robert Maxey
BEAD/ECL

rec'd 05/16/2000

RTI/7570/001/004-03 FR

May 2000

FINAL REPORT

REVIEW, EVALUATE, AND INDEPENDENTLY TEST SOIL AND WATER ENVIRONMENTAL CHEMISTRY METHODS (ECMs)

METHOD OF ANALYSIS OF THE DETERMINATION OF CGA 302371 (UE METABOLITE) IN SOIL BY LC/MS

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Contract Number: 68-W-98-208

Work Assignment Number: 2-4

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TABLE OF CONTENTS

SECTION 1.0

SUMMARY AND CONCLUSIONS

This report describes testing of a Registrant's method (MRID# 446461-02) for the determination of CGA-302371 in soil. The laboratory evaluation, major difficulties, experimental conclusions and comments are presented in this section.

1.1 LABORATORY EVALUATION

CGA-302371 recoveries ranged from 78.0% to 79.6% with a mean recovery of 78.7% and a relative standard deviation (RSD) of 0.9% for samples fortified at 0.005 ppm (LOQ). For samples fortified at 0.05 ppm (10xLOQ), the recoveries ranged from 82.4% to 85.5% with a mean recovery of 83.8% and an RSD of 1.6%. The instrument response to CGA-302371 in samples fortified at the method detection limit (LOD) exceeded noise by a factor greater than nine (S/N = 16.5). The LOD for CGA-302371 was 0.001 ppm.

No peaks were found in the retention time window of CGA-302371 in either the reagent blank or the matrix blank. The calibration data for the target analyte in samples were linear weighted (1/x) over the range of 0.000989 to 0.198 µg/mL (mean calibration factor RSD was 5.9% for the analyte, Appendix A). The calibration curve produced a correlation coefficient of 0.9988. The concentrations of the target analyte in the sample extracts were calculated using the response factors from the calibration curve. The retention times for the initial calibration standards and for all fortified samples ranged from 8.94 to 9.55 minutes for CGA-302371. Representative chromatograms are included in Appendix B.

The data presented in the Status Report 1 (Appendix C) was calculated based on the linear regression curve generated from the calibration data instead of response factors. The final report contains results from data calculated based on response factors as indicated in the method.

1.2 MAJOR DIFFICULTIES

Lab preparation activities or sample analysis did not present any problems.

1.3 CONCLUSIONS

Mean recoveries for soil samples fortified with CGA-302371 met acceptance criteria (70% to 120%) at both the LOQ and 10xLOQ fortification levels. The recovery RSD values were less than 20% for the analyte. Mean recovery values obtained by RTI for CGA-302371 were comparable to the values reported by the Registrant. Recovery RSD values reported by RTI were much lower than the values reported by the Registrant. The Registrant's data are presented below for comparison.

Reported Recovery and Precision From
Method Validation Results in Soils

Level	Analyte	Recovery Range (%)	Mean (%)	RSD (%)	n
LOQ[1]	CGA-302371	74.0 to 107	91.0	16	5
10XLOQ[2]	CGA-302371	72.0 to 96.0	80.0	12	5

[1]0.005 µg/g in soil, from Table 1 on page 15 of MRID# 446461-02.
[2]0.05 µg/g in soil, from Table 1 on page 15 of MRID# 446461-02.

1.4 COMMENTS

The time required for preparation of calibration and fortification standards was approximately 2 hours. The samples were extracted in sets with about 30 minutes difference between the start of each. One set included a matrix blank, solvent blank, and four fortified matrix samples (10xLOQ, LOQ or LOD). The entire extraction took about 7 hours. Samples can be analyzed overnight and data reduced the following day, which makes a total of approximately 3 working days to complete the project.

SECTION 2.0
ANALYTICAL RESULTS

This section presents method testing results. Summary tables are presented along with individual results from each sample at each spiking level.

2.1 DATA SUMMARY

	Accuracy and Precision Data		
Spike Level (µg/g)	Mean	SD[1]	RSD[2]
LOD (0.001) CGA-302371	83.4	3.4	4.1
LOQ (0.005) CGA-302371	78.7	0.7	0.9
10xLOQ (0.05) CGA-302371	83.8	1.3	1.6

[1]SD = Standard Deviation
[2]RSD = Relative Standard Deviation

2.2 ANALYTICAL SPIKE INFORMATION

Spike Level	Concentration of Spiking Solution (µg/mL)	Amount Spiked (mL)	Concentration in Sample (µg/g)	Sample W_i[1] (g)	Sample V_f[2] (mL)
LOD	0.198	0.100	0.000989	20.0	7.00
LOQ	0.989	0.100	0.00495	20.0	7.00
10xLOQ	9.89	0.100	0.0495	20.0	7.00

[1]W_i = Initial Sample Weight
[2]V_f = Final Sample Volume

2.3 INDIVIDUAL RESULTS AT THE LOD (0.000989 µg/g)

Sample Name	Retention Time (min)	Concentration Found (µg/g)	Conc. Fortified Sample (µg/g)
2-CGA-LOD1	8.94	0.000806	0.000989
2-CGA-LOD2	8.97	0.000793	0.000989
2-CGA-LOD3	8.95	0.000870	0.000989
2-CGA-LOD4	8.96	0.000831	0.000989

2.4 INDIVIDUAL RESULTS AT THE LOQ (0.00495 µg/g)

Sample Name	Retention Time (min)	Concentration Found (µg/g)	Conc. Fortified Sample (µg/g)	Percent Recovery
2-CGA-LOQ1	8.96	0.00386	0.00495	78.0
2-CGA-LOQ2	8.96	0.00391	0.00495	79.0
2-CGA-LOQ3	8.97	0.00394	0.00495	79.6
2-CGA-LOQ4	8.96	0.00388	0.00495	78.4

2.5 INDIVIDUAL RESULTS AT THE 10XLOQ (0.0495 µg/g)

Sample Name	Retention Time (min)	Concentration Found (µg/g)	Conc. Fortified Sample (µg/g)	Percent Recovery
2-CGA-10LOQ1	8.95	0.0408	0.0495	82.4
2-CGA-10LOQ2	8.98	0.0412	0.0495	83.2
2-CGA-10LOQ3	8.97	0.0416	0.0495	84.0
2-CGA-10LOQ4	8.96	0.0423	0.0495	85.5

SECTION 3.0
EXPERIMENTAL DETAILS

A brief summary of the analytical methods as performed by RTI and example calculations are presented in this section.

3.1 METHOD SUMMARY

A soil matrix was fortified with CGA-302371 at three different concentrations corresponding to the LOD, the LOQ, and ten times the LOQ. The fortification levels were: 0.001 ppm, 0.005 ppm, and 0.05 ppm, respectively. Four replicates at each concentration were prepared and analyzed according to the specified procedure. A linear regression (weighted (1/x) was used to characterize the relationship between standards of known concentrations and the detector response for the compound. The concentrations of the analyte in the sample extracts were calculated using the response factors of the calibration curve. The method protocol is described briefly below.

3.1.1 Sample Preparation

A 20 g aliquot of soil was weighed into a 250 mL polypropylene centrifuge bottle. Soil samples were fortified at this point with 100 µL of the appropriate fortification standard and allowed to evaporate and equilibrate for about 15 minutes. Twenty mL of 1:1 acetone/water, pH 3 solution was added to the soil and shaken via a platform shaker for about 30 minutes. The samples were shaken by hand and placed in a sonic bath for 10 minutes. The samples were centrifuged for at least 5 minutes at about 5000 RPM until separated. A 14.0 mL aliquot was removed and placed into a 16 mL test tube. The extract was concentrated to less than 7.0 mL using a nitrogen evaporator. The final volume was adjusted to 7.0 mL with water. Samples were filtered through 0.45 µm filters before transferring to HPLC vials for LC/MS/MS analysis.

3.1.2 Analysis Method

The soil samples were analyzed by liquid chromatography/mass spectrometry using a Perkin Elmer Series 200 Micro LC with an PE-Sciex API 365 triple quadrupole mass spectrometer. Operating conditions are listed below.

Instrumentation		
Liquid Chromatograph	Series 200 Micro (Perkin Elmer) (s/n 291N7013102)	
Autoinjector	Series 200 (Perkin Elmer) (s/n 293N8032704)	
Data System	MassChrom 1.1 Software (PE-Sciex)	
Operating Conditions		
Column	Symmetry C18, 5 µm, 250 x 4.6 mm i.d. (Waters)	
Mobile Phase A	Water with 0.2% acetic acid	
Mobile Phase B	Acetonitrile with 0.2% acetic acid	
Mode	Linear gradient	
Program	0% B to 50% B in 10 min; then 50% B to 100% B in 5 minutes	
Flow Rate	0.9 mL/min	
Injection Volume	100 µL	
Mass Spectrometer	API 365 triple quadrupole (PE-Sciex) (s/n C064300980)	
Heated Nebulizer Interface		
Nebulizer Temperature	475°C	
Auxiliary Flow	"4" setting	
Nebulizer Gas	nitrogen, 70 psi	
Detection Mode	positive ion Electrospray	
Interface Parameters		
Curtain Gas	"7" setting	
Ring Potential	100 V	
Orifice	30 V	
Collision Gas	nitrogen, "0" setting	
Detection Mode	Selected Ion Monitoring (SIM)	
Ions Monitored (m/z)	Quantitation Ion	148
	Qualifier Ion	150

Standard Information: CGA-302371
Novartis Crop Protection, Inc.
DAH-XXIV-80
Neat, 99.1% Pure
Received: 1/20/00
Prepared: 1/31/00

Matrix Information: Soil
Provided by Iowa Soil, Boone Co. Webster
Lot 2
Collected 9/16/98
Received 11/4/98

3.2 CALCULATIONS

Example calculations are presented for response factors, concentration, and percent recovery. The examples are taken from the analysis of CGA-302371 in a soil sample (replicate 1) fortified at 0.0495 ppm.

3.2.1 Response Factor (RF)

$$RF = \frac{Concentration\ of\ Standard\ (ppm)}{Peak\ Area\ of\ Standard}$$

For calibration curve point 0.198 ppm, the peak area = 173988.453

$$RF = \frac{0.198}{173988.453} = 1.1380E\text{-}06$$

3.2.2 Concentration (ppm) of Analyte in Soil

$$Conc.\ (ppm) = \frac{(Peak\ Area\ x\ Mean\ RF)\ x\ FV}{g.Extracted}\ x\ AF$$

where: Conc. (ppm) = Concentration of analyte in µg/g (ppm)

FV = Final sample volume (mL)

g.Extracted = initial grams of sample extracted (wet weight)

$$AF = Aliquot\ Factor = \frac{Extraction\ volume\ (mL)}{Aliquot} = \frac{20\ mL}{14\ mL}$$

Mean RF = Mean of response factor from calibration curve

NOTE: Residues are calculated based on the wet weight of soil and are not corrected for moisture content.

For replicate 1 of the 10xLOQ sample, the peak area = 65995.25, Mean RF = 1.2351E-06, FV = 7 mL, g.Extracted = 20 g.

$$Conc.\ (ppm) = \frac{(65995.25\ x\ 1.2352E\text{-}06)\ x\ 7}{20}\ x\ \frac{20}{14}$$

$$Conc.\ (ppm) = 0.0408$$

3.2.3 <u>% Recovery</u>

$$\%\ Recovery = \frac{Recovery\ Level\ (ppm)}{Fortification\ Level\ (ppm)}\ x\ 100$$

Recovery Level = the recovery found in the spiked control (ppm)

For replicate 1 (10xLOQ), recovery level = 0.0408 ppm and fortification level = 0.0495 ppm.

$$\%\ Recovery = \frac{0.0408}{0.0495}\ x\ 100$$

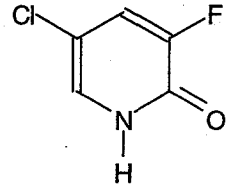
$$\%\ Recovery = 82.4$$

3.3 CHEMICAL STRUCTURE DIAGRAMS OF CGA-302371

Chemical Name: 5-Chloro-3-fluoro-1.H.pyridin-2-one

CAS #: Not Available

Source: Novartis Crop Protection



APPENDIX A - CALIBRATION DATA

A.1 Initial Eight-Point Calibration Data for CGA-302371

n	Concentration (µg/mL)	Area Counts	RF
1	0.000989	763.364	1.2956e-06
2	0.00248	2228.530	1.1128e-06
3	0.00495	3856.842	1.2834e-06
4	0.00989	7690.390	1.2860e-06
5	0.0495	38542.984	1.2843e-06
6	0.0989	78057.836	1.2670e-06
7	0.148	121906.133	1.2140e-06
8	0.198	173988.453	1.1380e-06

Mean Response Factor = 1.2352e-06

Standard Deviation = 7.2547e-08

Relative Standard Deviation = 5.9

APPENDIX B - REPRESENTATIVE CHROMATOGRAMS

This section contains representative chromatograms of the reagent blank, matrix blank, calibration standards, and spiked samples at each fortification level in the following order:

- Matrix blank
- Reagent blank
- Samples at the LOD (0.000989 ppm)
- Samples at the LOQ (0.00495 ppm)
- Samples at the 10xLOQ (0.0495 ppm)
- Calibration Standards
- Signal/noise response

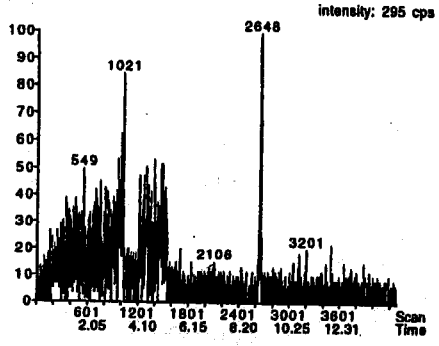
MacQuan, version 1.6
Printed: Thu, Feb 24, 2000 11:13
Calibration File: CAL2_23SOLVENT Path: Macintosh HD: MassChrom 1.1: Project Files:nora:
Comments: SOLVENT STD QUANITATION

7570-4-72 .001 std Wed, Feb 23, 2000 17:52
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	811.775
Height	270
Start Time	8.97
End Time	9.11
Integration Width	0.15
Retention Time	9.05
Integration Type	M



7570-4-74 .0025 STD Wed, Feb 23, 2000 18:26
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

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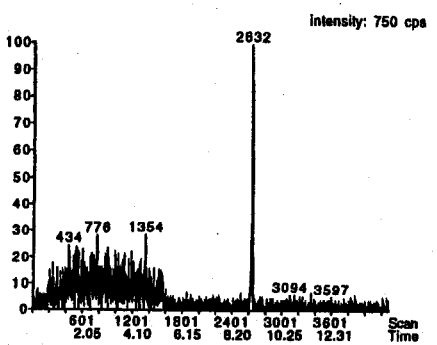
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	2490.181
Height	727
Start Time	8.91
End Time	9.06
Integration Width	0.15
Retention Time	8.99
Integration Type	M



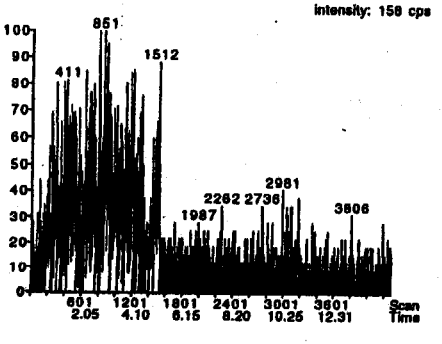
7570-4-73 2-CGA-MBLK LOD Wed, Feb 23, 2000 18:09
No Comment

Matrix Blank

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	0.0
Height	0
Start Time	0.00
End Time	0.00
Integration Width	0.00
Retention Time	0.00
Integration Type	



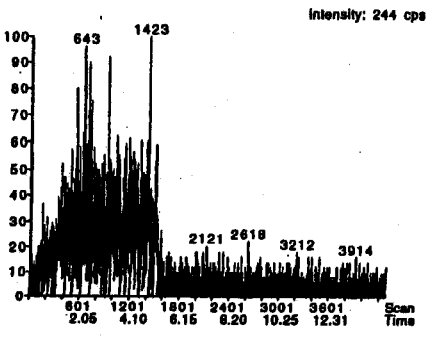
7570-4-75 2-CGA-SBLK LOD Wed, Feb 23, 2000 18:44
No Comment

Reagent Blank

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	0.0
Height	0
Start Time	0.00
End Time	0.00
Integration Width	0.00
Retention Time	0.00
Integration Type	



7570-4-76 .005 STD Wed, Feb 23, 2000 19:01
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

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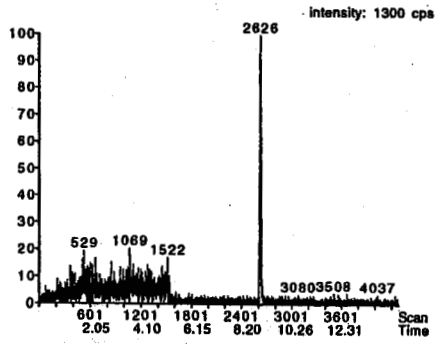
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	4546.273
Height	1293
Start Time	8.90
End Time	9.08
Integration Width	0.17
Retention Time	8.97
Integration Type	M



7570-4-78 2-CGA-LOD2 Wed, Feb 23, 2000 19:35
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

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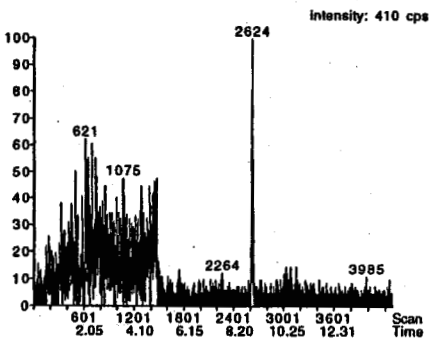
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	1284.232
Height	410
Start Time	8.90
End Time	9.05
Integration Width	0.15
Retention Time	8.97
Integration Type	M

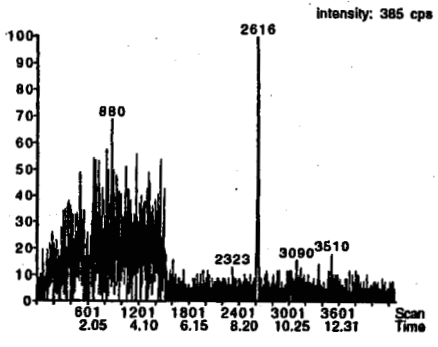


7570-4-77 2-CGA-LOD1 Wed, Feb 23, 2000 19:18
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	1305.218
Height	378
Start Time	8.89
End Time	9.02
Integration Width	0.13
Retention Time	8.94
Integration Type	M



7570-4-79 2-CGA-LOD3 Wed, Feb 23, 2000 19:53
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

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148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
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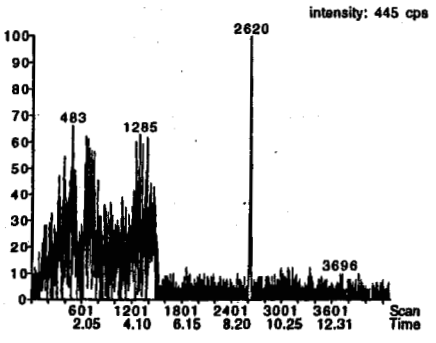
Height	437
Start Time	8.88
End Time	9.03
Integration Width	0.15
Retention Time	8.95
Integration Type	M



7570-4-80 2-CGA-LOD4 Wed, Feb 23, 2000 20:10
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

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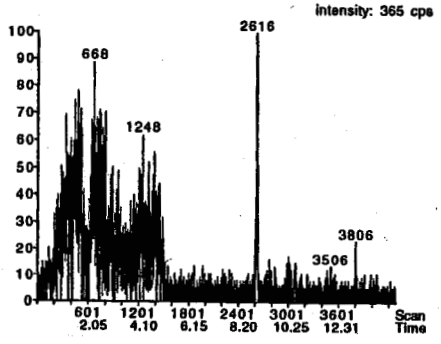
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	1345.104
Height	358
Start Time	8.88
End Time	9.03
Integration Width	0.15
Retention Time	8.96
Integration Type	M



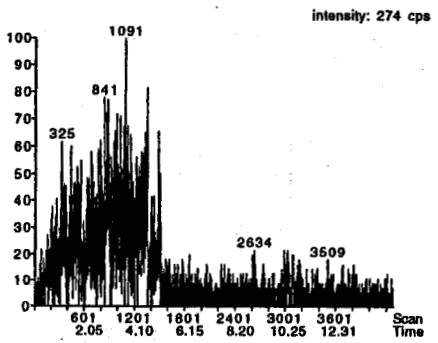
7570-4-82 2-CGA-MBLK LOQ Wed, Feb 23, 2000 20:45
No Comment

Matrix Blank

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	0.0
Height	0
Start Time	0.00
End Time	0.00
Integration Width	0.00
Retention Time	0.00
Integration Type	

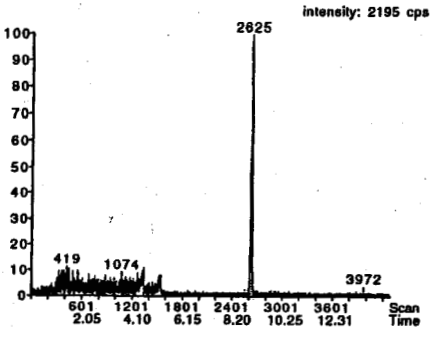


7570-4-81 .01 STD Wed, Feb 23, 2000 20:27
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	8469.655
Height	2172
Start Time	8.87
End Time	9.06
Integration Width	0.18
Retention Time	8.97
Integration Type	M



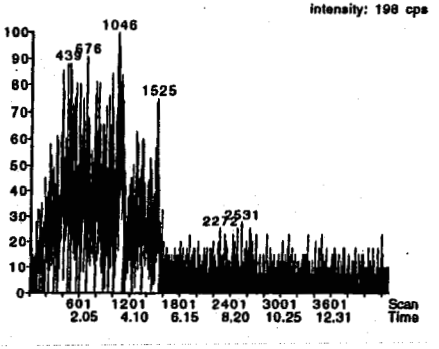
7570-4-83 2-CGA-SBLK LOQ Wed, Feb 23, 2000 21:02
No Comment

Reagent Blank

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	0.0
Height	0
Start Time	0.00
End Time	0.00
Integration Width	0.00
Retention Time	0.00
Integration Type	

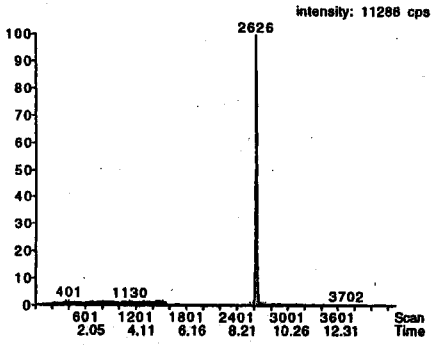


7570-4-84 .05 STD Wed, Feb 23, 2000 21:19
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	44834.969
Height	11285
Start Time	8.89
End Time	9.22
Integration Width	0.33
Retention Time	8.97
Integration Type	M

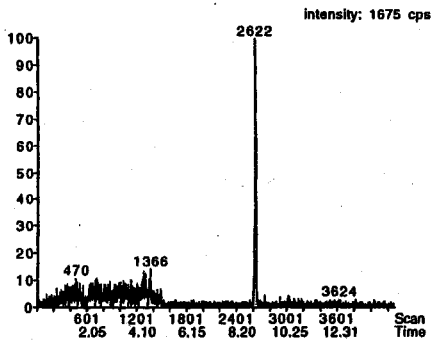


7570-4-86 2-CGA-LOQ2 Wed, Feb 23, 2000 21:54
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	6325.169
Height	1656
Start Time	8.89
End Time	9.06
Integration Width	0.17
Retention Time	8.96
Integration Type	M

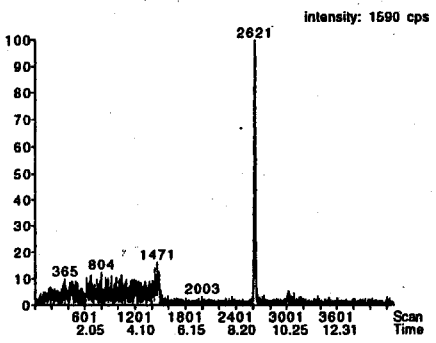


7570-4-85 2-CGA-LOQ1 Wed, Feb 23, 2000 21:37
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	6246.476
Height	1575
Start Time	8.91
End Time	9.09
Integration Width	0.18
Retention Time	8.96
Integration Type	M

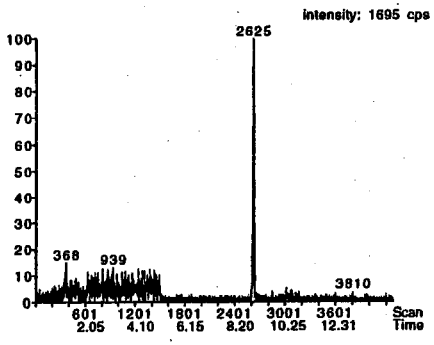


7570-4-87 2-CGA-LOQ3 Wed, Feb 23, 2000 22:11
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	6378.140
Height	1675
Start Time	8.89
End Time	9.02
Integration Width	0.13
Retention Time	8.97
Integration Type	M

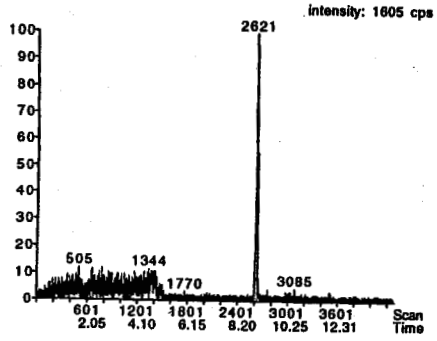


7570-4-88 2-CGA-LOQ4 Wed, Feb 23, 2000 22:29
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	6275.394
Height	1590
Start Time	8.87
End Time	9.07
Integration Width	0.20
Retention Time	8.96
Integration Type	M



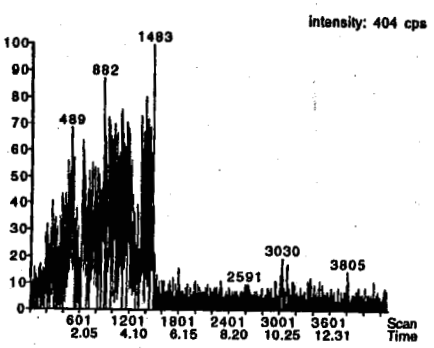
7570-4-90 2-CGA-MBLK 10 LOQ Wed, Feb 23, 2000 23:03
No Comment

Matrix Blank

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	0.0
Height	0
Start Time	0.00
End Time	0.00
Integration Width	0.00
Retention Time	0.00
Integration Type	

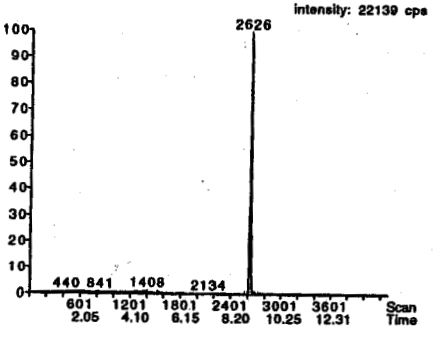


7570-4-89 .1 STD Wed, Feb 23, 2000 22:46
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	90129.063
Height	22139
Start Time	8.89
End Time	9.22
Integration Width	0.33
Retention Time	8.97
Integration Type	M



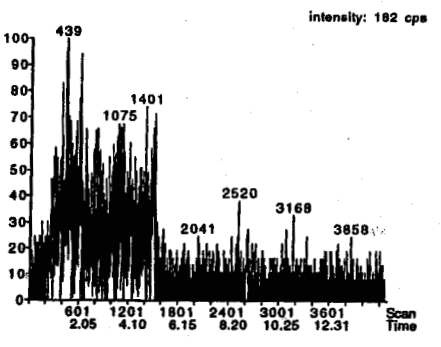
7570-4-91 2-CGA-SBLK LOQ Wed, Feb 23, 2000 23:21
No Comment

Reagent Blank

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	0.0
Height	0
Start Time	0.00
End Time	0.00
Integration Width	0.00
Retention Time	0.00
Integration Type	

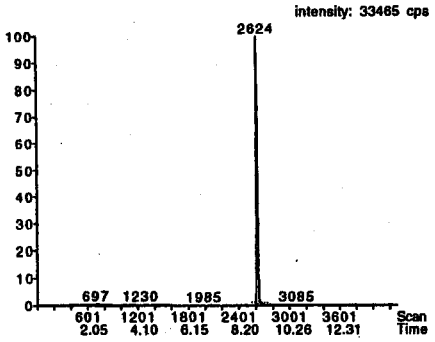


7570-4-92 .15 STD Wed, Feb 23, 2000 23:38
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	140003.438
Height	33464
Start Time	8.87
End Time	9.25
Integration Width	0.38
Retention Time	8.97
Integration Type	M

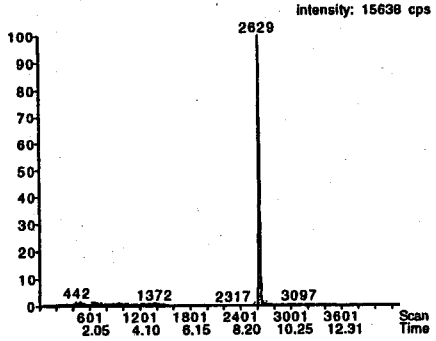


7570-4-94 2-CGA-10LOQ2 Thu, Feb 24, 2000 00:13
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	66744.703
Height	15631
Start Time	8.88
End Time	9.16
Integration Width	0.28
Retention Time	8.98
Integration Type	M

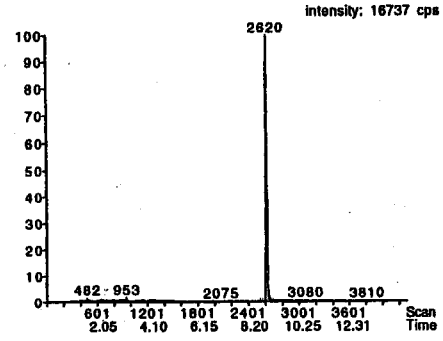


7570-4-93 2-CGA-10LOQ1 Wed, Feb 23, 2000 23:55
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	65995.250
Height	16726
Start Time	8.82
End Time	9.11
Integration Width	0.29
Retention Time	8.95
Integration Type	M

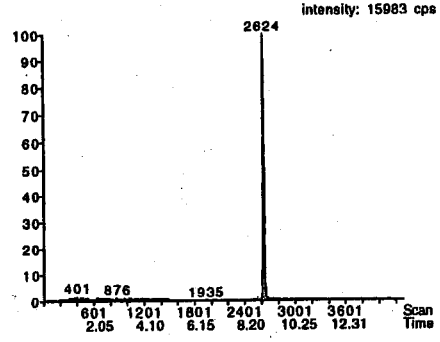


7570-4-95 2-CGA-10LOQ3 Thu, Feb 24, 2000 00:30
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	67357.375
Height	15973
Start Time	8.85
End Time	9.31
Integration Width	0.46
Retention Time	8.97
Integration Type	M

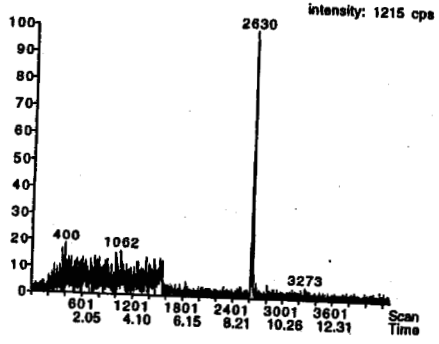


7570-4-065 .005 STD Wed, Feb 23, 2000 15:24
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	3856.842
Height	1194
Start Time	8.90
End Time	9.07
Integration Width	0.18
Retention Time	8.99
Integration Type	M

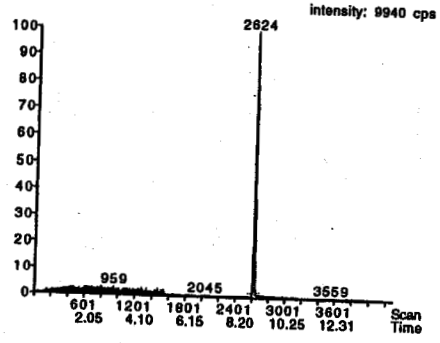


7570-4-067 .05 STD Wed, Feb 23, 2000 15:58
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	38542.984
Height	9899
Start Time	8.89
End Time	9.10
Integration Width	0.21
Retention Time	8.97
Integration Type	M

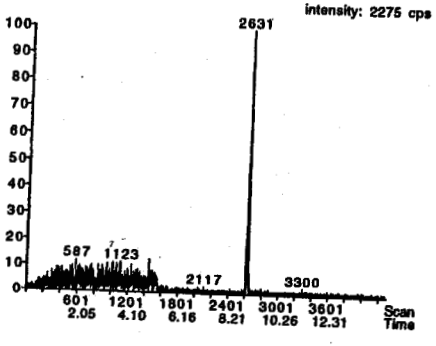


7570-4-066 .01 STD Wed, Feb 23, 2000 15:41
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	7690.390
Height	2255
Start Time	8.92
End Time	9.09
Integration Width	0.17
Retention Time	8.99
Integration Type	M

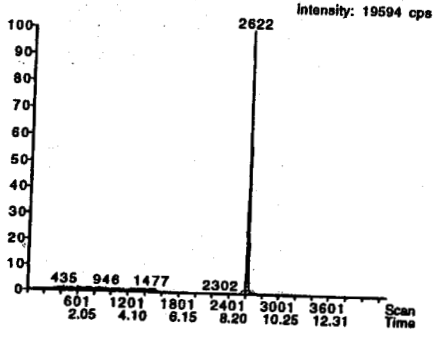


7570-4-068 .1 STD Wed, Feb 23, 2000 16:16
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	78057.836
Height	19560
Start Time	8.86
End Time	9.16
Integration Width	0.30
Retention Time	8.96
Integration Type	M



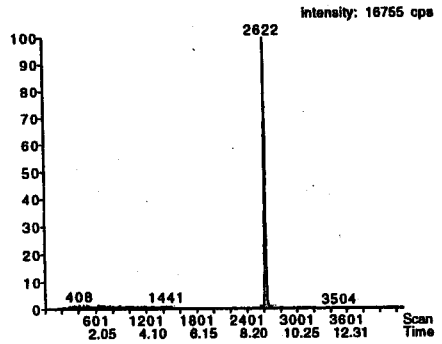
MacQuan, version 1.6
Printed: Thu, Feb 24, 2000 11:13
Calibration File: CAL2_23SOLVENT Path: Macintosh HD: MassChrom 1.1: Project Files:nora:
Comments: SOLVENT STD QUANITATION

7570-4-96 **2-CQA-10LOQ4** Thu, Feb 24, 2000 00:47
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	68545.094
Height	16752
Start Time	8.87
End Time	9.28
Integration Width	0.42
Retention Time	8.96
Integration Type	M

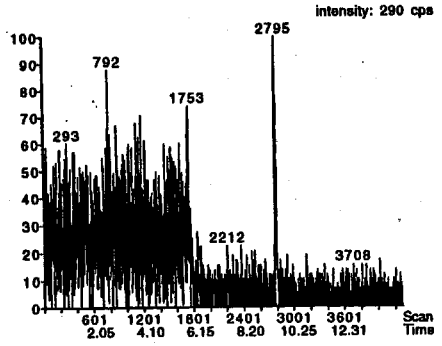


7570-4-063 **.001 STD** Wed, Feb 23, 2000 14:49
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	763.364
Height	270
Start Time	9.48
End Time	9.61
Integration Width	0.12
Retention Time	9.55
Integration Type	M

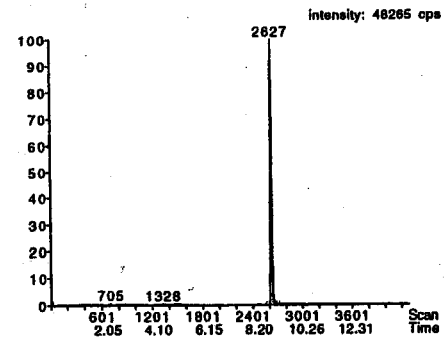


7570-4-97 **.2 STD** Thu, Feb 24, 2000 01:05
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	194955.500
Height	48261
Start Time	8.89
End Time	9.26
Integration Width	0.38
Retention Time	8.98
Integration Type	M

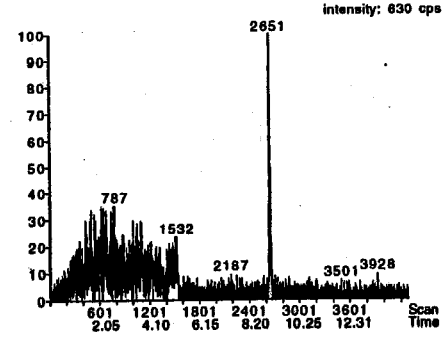


7570-4-064 **.025 STD** Wed, Feb 23, 2000 15:07
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	2226.530
Height	620
Start Time	8.98
End Time	9.13
Integration Width	0.15
Retention Time	9.06
Integration Type	M

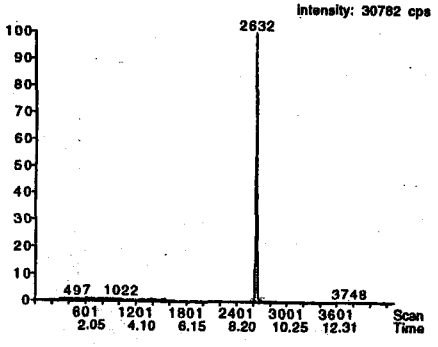


7570-4-069 .15 STD Wed, Feb 23, 2000 16:33
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	121906.133
Height	30769
Start Time	8.91
End Time	9.17
Integration Width	0.27
Retention Time	8.99
Integration Type	M

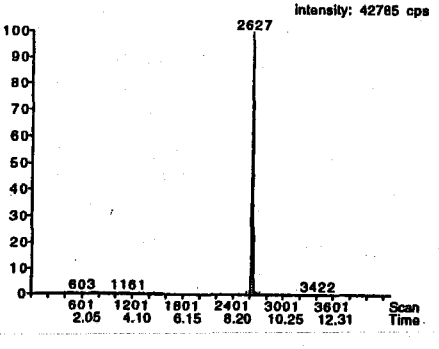


7570-4-070 .2 STD Wed, Feb 23, 2000 16:50
No Comment

14.63 in 1 period
MSMS ION
No Internal Standard
Use Area

1: 14.64 MRM, 4285 scans
148.0->129.9

Noise Thres.	10.0
Quant Thres.	2.0
Min. Width	6
Mult. Width	2
Base. Width	100
RT Win. (secs)	20
Smooth	0
Expected RT	8.95
Area	173988.453
Height	42773
Start Time	8.89
End Time	9.19
Integration Width	0.30
Retention Time	8.98
Integration Type	M

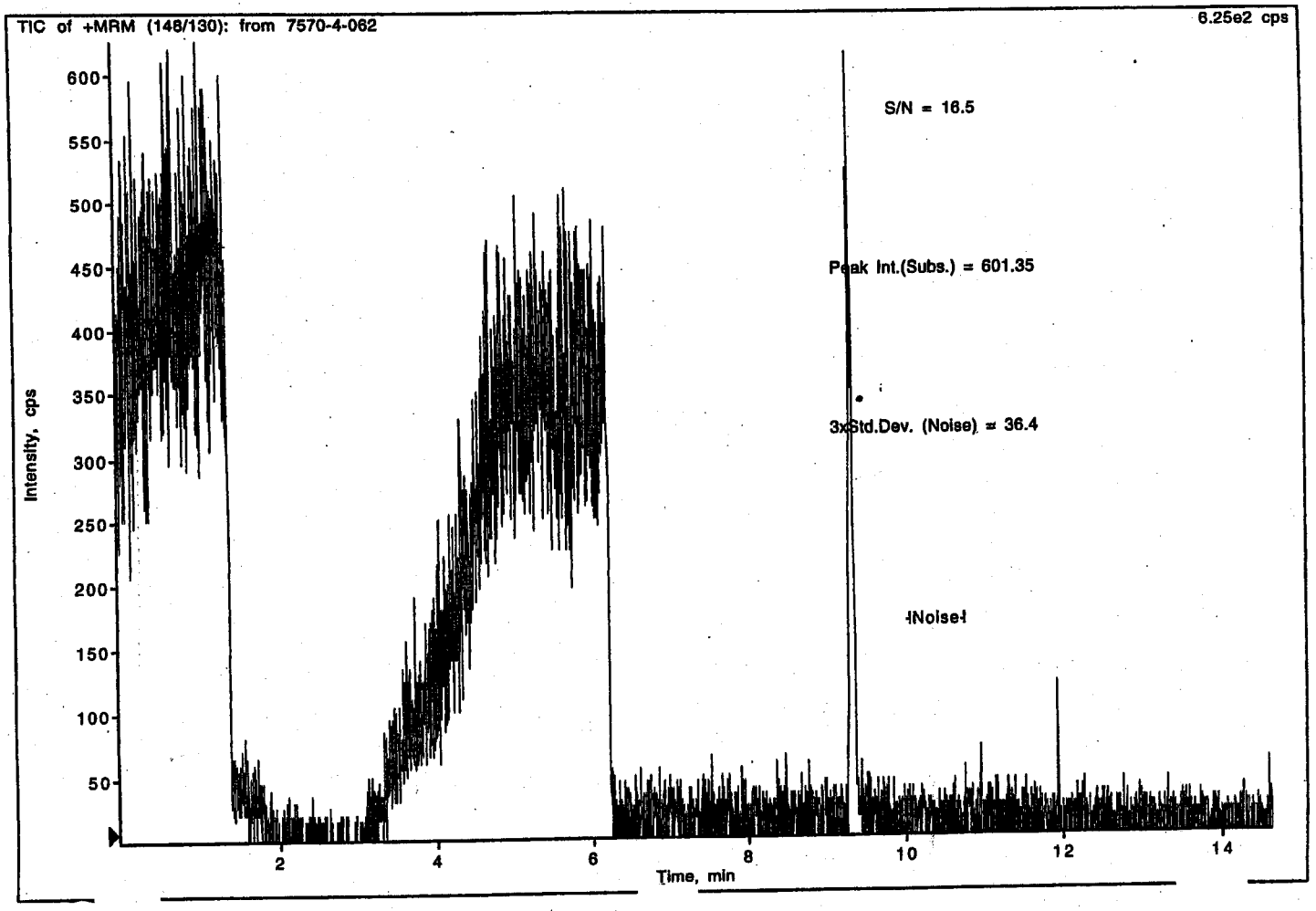


MultiView 1.4 Wednesday, February 23, 2000 14:33 page 1 of 1

7570-4-062

Period 1, Expt. 1; Dwell: 200.0 ms; Pause: 5.0 ms

Acq. Time: Wed, Feb 23, 2000 at 14:16:00; Exp. Comment: CGA 147.0 MRM Experiment



APPENDIX C - STATUS REPORT 1

STATUS REPORT No. 1

Method:	Method of Analysis of the Determination of CGA 302371 (UE Metabolite) in Soil by LC/MS
Analyte:	CGA-302371
Date:	3/1/00
MRID No.:	446461-02
ECM No.:	ECM0162S1
Status Report No.:	1
RTI Chemist	Nora P. Castillo

Purpose

The purpose of this document is to report the results of the laboratory evaluation for the method ECM0162S1.

Results from Evaluation

CGA-302371 recoveries ranged from 80.0 to 83.1% with a mean recovery of 81.4% and a relative standard deviation of 1.6% (N=4) for samples fortified at 0.0495 µg/ml (ppm) (10xLOQ). For samples fortified at 0.00495 µg/ml (LOQ), the recoveries ranged from 76.8 to 78.4% with a mean recovery of 77.5% and a relative standard deviation of 0.9% (N=4). For samples fortified at 0.000989 µg/ml (LOD), the recoveries ranged from 83.9 to 91.4% with a mean recovery of 87.0% and a relative standard deviation of 3.8% (N=4). See Table 1 for all recoveries.

The calibration data for the target compound was linear over the range of 0.000989 to 0.198 µg/ml, with r = 0.9988. Operating conditions are shown in Table 2.

Problems Encountered

Lab preparation activities or sample analysis did not present any problems.

Critical Review of the Method

The method was extracted in three sets with about 30 minutes difference between the start of each. One set included a matrix blank, solvent blank, and four fortified matrix samples (10xLOQ, LOQ or LOD). The entire extraction took about 7 hours.

Time Frame

These experiments may be prepared in one day and analyzed the following day with a maximum time of three days.

Table 1. Results from Evaluation

Sample ID	MS File ID	Nominal Fortif. Level (ppm)	Found Conc. (µg/ml)	Found Conc. (ppm)	Method Recovery %
2-CGA-MBLK-LOD	7570-4-73	0	0	0	0
2-CGA-SBLK-LOD	7570-4-75	0	0	0	0
2-CGA-LOD1	7570-4-77	0.00989	0.001684	0.000842	85.1
2-CGA-LOD2	7570-4-78	0.00989	0.001659	0.000830	83.9
2-CGA-LOD3	7570-4-79	0.00989	0.001808	0.000904	91.4
2-CGA-LOD4	75750-4-80	0.00989	0.001732	0.000866	87.6
				Mean ± SD	87.0 ± 3.32
				(RSD)	(3.8)
				Recov. Range	83.9 - 91.4
2-CGA-MBLK-LOQ	7570-4-82	0	0	0	0
2-CGA-SBLK-LOQ	7570-4-83	0	0	0	0
2-CGA-LOQ1	7570-4-85	0.00495	0.007606	0.00380	76.8
2-CGA-LOQ2	7570-4-86	0.00495	0.007700	0.00385	77.8
2-CGA-LOQ3	7570-4-87	0.00495	0.007763	0.00388	78.4
2-CGA-LOQ4	7570-4-88	0.00495	0.007640	0.00382	77.2
				Mean ± SD	77.5 ± 0.70
				(RSD)	(0.9)
				Recov. Range	76.8 - 78.4
2-CGA-MBLK-10LOQ	7570-4-90	0	0	0	0
2-CGA-SBLK-10LOQ	7570-4-91	0	0	0	0
2-CGA-10LOQ1	7570-4-93	0.0495	0.07921	0.0396	80.0
2-CGA-10LOQ2	7570-4-94	0.0495	0.08011	0.0401	80.9
2-CGA-10LOQ3	7570-4-95	0.0495	0.08084	0.0402	81.7
2-CGA-10LOQ4	7570-4-96	0.0495	0.08226	0.0411	83.1
				Mean ± SD	81.4 ± 1.31
				(RSD)	(1.6)
				Recov. Range	80.0 - 83.1

Table 2. Instrumental Parameters

Instrumentation		
Liquid Chromatograph	Series 200 Micro (Perkin Elmer) (s/n 291N7013102)	
Autoinjector	Series 200 (Perkin Elmer) (s/n 293N8032704)	
Data System	MassChrom 1.1 Software (PE-Sciex)	
Operating Conditions		
Column	Symmetry C18, 5 μm, 250 x 4.6 mm i.d. (Waters)	
Mobile Phase A	Water with 0.2% acetic acid	
Mobile Phase B	Acetonitrile with 0.2% acetic acid	
Mode	Linear gradient	
Program	0% B to 50% B in 10 min; then 50% B to 100% B in 5 minutes	
Flow Rate	0.9 mL/min	
Injection Volume	100 μL	
Mass Spectrometer	API 365 triple quadrupole (PE-Sciex) (s/n C064300980)	
Heated Nebulizer Interface		
Nebulizer Temperature	475°C	
Auxiliary Flow	"4" setting	
Nebulizer Gas	Nitrogen, 70 psi	
Detection Mode	Positive ion Electrospray	
Interface Parameters		
Curtain Gas	"7" setting	
Ring Potential	100 V	
Orifice	30 V	
Collision Gas	Nitrogen, "0" setting	
Detection Mode	Selected Ion Monitoring (SIM)	
Ions Monitored (m/z)	Quantitation Ion	148
	Qualifier Ion	150